THE EXAMINATION OF BLOOD STAINS IN

MEDICO-LEGAL CASES:

An investigation into the efficiency of existing tests and the spectroscopic differentiation of dyes and other pigments.

Thesis for the degree of M.D. Edin.

by

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The examination of blood stains being one of the most common procedures in Medico-legal practice, it is of the highest importance that the technique for such examination should be as simple as possible and the results uniformly certain, so that it can be definitely stated in every case whether a certain stain does or does not contain blood. In the early part of last century there were no tests for blood, the observers of that period depending entirely on the examination of the colour of suspected stains. In 1817 Orfila, who was the leading chemist of his day, brought forward some so-called specific tests for blood; none of these tests (seven in all) depended on the presence of haemoglobin, however, but were simply tests for albumen and, though useful as such, were quite inadmissable as tests for blood. His work on the chemistry of the blood stimulated further inquiry and great numbers of tests were advocated by different workers, but none of these were of sufficient value to stand the test of time until, in 1853, Teichmann reported that when blood was treated with Glacial Acetic Acid in the presence of a trace of common salt and heated, typical brown rhombic crystals were produced. This test remains to the present day as one of the most definite tests for blood.

In 1861 Van Deen reported that when a solution of blood/

blood was treated with Tincture of Guaiacum and old oil of Turpentine a blue colour was obtained and he considered this to be specific. This test was critically examined by Liman of Berlin, who believed in its specificity. Dr. Day of Geelong (Aust. Med. Journ., May 1867) further improved the test by substituting H_2O_2 in Ether for the Turpentine and after a large series of experiments stated that the results were highly satisfactory. Taylor (Principles of Med., June 1873) stated that during tests extending over six years he met no obstacle to the detection of blood by this method.

The test depends on the presence in the stain of a catalytic agent which acts as a carrier of Oxygen from the Hydrogen Peroxide to the Guaiacum which is thus oxidised to Guaiaconic Acid and finally to Guaiac Blue. This agent is present not only in blood but in numbers of organic substances, moreover many inorganic bodies act in a similar manner, so that the test is useless for medico-legal purposes.

In 1862 Hoppe Seyler investigated the optical properties of blood pigment and described its characteristic absorption bands in the solar spectrum.

Further work on this test and on Teichmann's crystal test has made the examination of blood absolutely conclusive. In recent years the advent of/

of the Biological Tests has greatly increased our powers in differentiating the blood of different animals, but as the latter are specific tests for albumen and not for blood it is as much as ever necessary to have definite tests for blood. Moreover, since many experiments may have to be made with the stain extracts in the Biological Tests, it is more than ever necessary to be able to apply our tests for haemoglobin to as small a quantity of material as possible, and if one could apply all the necessary tests to one minute portion of the suspected stain it would be a great saving in time and material.

In no English or American Text Book is the subject dealt with in a satisfactory manner, the technique for the Teichmann Test is given in a form which must lead to many errors, the difficulties in the way of the test are scarcely mentioned and no hints given for surmounting them. The explanation of the spectroscopic tests is laboured and very confusing. No indication is given of the relative value and delicacy of the tests and such spectra as acid and alkaline haematin, methaemoglobin, sulphaemoglobin, etc., which are the reverse of delicate and which are not characteristic, are given as much prominence as that of haemochromogen. The spectra of Acid and Alk Haematoporphyrin which are most delicate and characteristic tests are in many cases not mentioned, while in those/

those works where they are quoted, either no method is given for their formation, or as in other cases the technique is most confusing and complicated and would require large quantities of blood for the test.

In the Continental works (Leers, Wachholz, Puppe, Balthazard, etc.) much more prominence is given to the subject, but here again are innumerable errors, due to faulty technique, especially in regard to the Teichmann Test; and moreover these works are not always available to the English reader.

The object of the present inquiry is, by investigation of the value of the various tests applied to blood under the most diverse conditions, to formulate a series of tests which will be absolutely conclusive in every case, to simplify and improve the technique of such examination so that all the tests can be applied to one minute fragment, and to render the solution of the stain, which is always tiresome and often impracticable, quite unnecessary.

Teichmann Test.

I have already mentioned that this test was first advocated by Teichmann in 1853 and have given a brief account of the original technique. A great many modifications of the original technique have been devised, none of which offer any special advantage except/

except the one which I shall mention and discuss later. The object in all is to convert the haemoglobin of the stain into haematin which, in the presence of a halogen, is converted into a halogen salt and crystallyzes out from the solution in brown rhombic crystals which are very characteristic. Wachholz states (Untersuch. über Häminkystalle; Vierteljahr f. Ger. Med. 3. 21. 2.) that it is immaterial whether the acid be Acetic, Lactic, Propionic, Butyric, Oxalic, Salicylic - H_2SO_4 HCl HNO_3 H_3PO_4- in concentrated solution of alcohol, 90-95%. He prefers a solution of Sulphuric Acid 1-10,000 in alcohol on account of the lower boiling point of this medium. Leers agrees with this. Nencki and Sieber use HCl in Amyl alcohol. Symons (Biochemical Journal, Dec. 1913. 597.) recommends Sod. Iodid. 1% in Glacial Acetic. Nippe (Deut. Med. Woch. Nov. 21, 12) pointed out that a solution of the halogens in Acetic enabled one to obtain crystals in cases where none were obtained by the ordinary method.

We have used here a solution of Potass Iod. - Chlorid. and Bromid. each .1 gram. in 100 c.c. Glacial Acetic Acid and find this to give constant and reliable results if properly used, and to have distinct advantages over the other modifications. The solution gradually acquires a red colour due to the liberation of Iodine, but this seems to increase its efficiency which/

which remains for a very long period. The crystals obtained by this method are of a darker colour and more obvious on examination.

The material for examination should be dry; if on Wood, Paper, Glass, Leather, etc., merely scrape off a few particles with the point of a clean knife. Knives, Razors, etc., should be carefully examined with a hand lens and a portion which has the dull brown lustre of blood carefully picked off, careless scraping will remove more iron rust than blood and make the test more difficult. In the joint of clasp knives, the name stamp, and the nail recess one can usually find blood even in a wiped knife. On fabrics, if the stain be on the surface, some dust can be readily scraped off. If, however, the stain be faint or partially washed out, a few fibres must be cut out and carefully teased out on the slide. Add a drop of the Acetic solution to the fragment on the slide, cover with a coverslip and heat gently over a low flame, holding the slide in the fingers to prevent overheating.

When a few bubbles appear remove from the source of heat and allow them to escape gently. Repeat this three or four times never allowing brisk ebullition to take place, cool and examine with the microscope, when the typical crystals will be readily seen with the low power.

Typical/

Typical crystals are brownish-red, lustrous rhombs; they vary greatly in size. Numbers are found lying singly in the field, while others aggregate into clusters, rosettes, etc. In numbers of cases there is a V-shaped depression at each end of the crystal.

Typical haemin crystals as seen under low power. (x 160)

Haemin crystals as observed under high power. (x 480)

Haemin crystals in woollen garment.

Crystals in blood from linen garment.

<u>Atypical crystals.</u> Dumb-bell and wheat-stone crystals are often the result of over-heating, they are also more often obtained from very old exposed stains.

In recent stains the crystals are first seen at the periphery of the clot and in great abundance, in old stains they are frequently only in the middle of the fragment and very few in number.

If the slide has a granular appearance but no crystals, it requires re-heating.

In cases where great difficulty is experienced in obtaining crystals, Richter recommends that the solution be left in contact with the stain for twenty-four hours before heating; in my experience, when crystals are not obtained at once by the method noted, they cannot be obtained by any other method.

The thinner the film and the more slowly the heating the larger are the crystals obtained.

Haematin crystals give no spectrum, they are Pleochroismic in the Micropolarization apparatus (Leers, Blut Untersuchung, Berlin 1910.) Leers states that they are permanent only when air is excluded as when mounted in Canada Balsam. I have exposed crystals in the window of the laboratory for three months without cover of any sort and they remain quite unaffected.

Reaction/

Reactions.

If the crystals be incinerated, treated with HCl and ammonium sulphocyanide, the red colour of Iron sulphocyanide is obtained.

Treated with H_2O_2 a brisk evolution of oxygen is at once observed.

They can be readily converted into Haemochromogen in a manner that I shall indicate later. They are insoluble in Glacial Acetic Acid.

Strong Sulphuric Acid is said to readily dissolve them. This is incorrect.

On the first addition of the acid a purple colour appears which gives the spectrum of acid haemato-porphyrin. On warming, the purple colour gets darker and the crystals float out into the acid, the blood clot is broken down and gradually disappears, leaving the crystals dark and distinct against a clear back-ground.

I have examined preparations after eight days and the crystals were still visible. Also after warming with H_2SO_4 and keeping at 37°C for six days, no change was observed in the crystals. Of course vigorous heating destroys the crystals as it will destroy any organic matter, but this can scarcely be called solution.

HNO_3. Readily soluble in cold.

On the addition of strong Nitric Acid, there is a vigorous/

vigorous evolution of gas, the fragment of clot becomes yellow and the crystals, triturated by the escaping gas, become semi-transparent in five minutes, and in eight minutes have disappeared.

HCl. Insoluble in cold and with moderate heating, although the blood clot disappears.

KOH. Alkalies are stated in every text book to which I have had access to readily dissolve the crystals. I cannot agree with this.

30% solution of Caustic Soda does not dissolve the crystals in the cold or with moderate heating. In five days at 37°C complete solution is effected. Alkali tends to decolourize the crystals and I think that this has led to the report that it readily dissolves them. They are not readily soluble and old crystals are comparatively insoluble.

They are insoluble in alcohol. Insoluble in ether, petroleum ether, chloroform and xylol.

Experimental.

Fresh specimens of blood from the following animals were examined to see if the crystals were obtained with equal facility in different classes of animals:- Human. Ox. Sheep. Rabbit. Pigeon. Fish. In every case crystals were obtained rapidly and with ease.

Blood/

Blood stains from the following specimens were examined to note the effect of time and exposure, and the effect of the different substances on which blood is found:-

1. Blood from Meningeal Haemorrhage exposed in Museum for 25 years. A fragment of the dry material gave abundant haematin crystals.
2. Fragment of blood from blade of razor (suicide 26. 5. 78) exposed for 36 years. Numerous small crystals.
3. Iron blade of table knife (murder and suicide 2. 11. 06). Large crystals - scanty but definite.
5. Fragment, .4 m.m. diameter, from blade of razor (26. 3. 03). Abundant typical crystals.
6. From bone handle of same. A few isolated crystals.
7. Menstrual blood from cotton garment exposed seven years. Portion of dry fibres gives abundant crystals.
8. Ditto after dissolving in water and drying film. Crystals readily.
9. Iron blade of razor exposed six years in Laboratory (much rust). Crystals badly formed, very few typical.
10. Razor (murder and suicide 16. 2. 99). Numerous haematin crystals and numbers of crystals and masses of debris like Haemochromogen crystals (no spectrum).

12. Razor (suicide 30. 4. 00), fragment from blade. Abundant small crystals, many typical haematin, many irregular forms. Mostly in centre, none at periphery.

13. Human Blood exposed in thin layer on Tin in window of Laboratory for four months. Abundant crystals at once.

14. Ditto on Paper = Crystals readily.

15. " " glass = " "

16. " " wood = " "

17. " " steel knife = " "

18. Dried fish blood found on pavement in street. Well formed crystals immediately.

19. Tweed cloth stained with Ox Blood in September 1908. Cloth stiff and reddish brown - some dust scraped off gave typical crystals in abundance.

20. Flannel stained with Ox Blood in September 1908. Reddish dust on scraping - no spectrum. Abundant haemin crystals obtained.

21. Green tweed ditto. Fabric stiff, no colour of stain visible. Crystals readily from powder scraped off surface.

22. Blue Vicuna cloth smeared with human blood September 1908. Cloth stiff, darker in colour. Crystals readily.

23. Light Homespun cloth stained with human serum January 1908. Cloth stiff. Yellowish brown stain. Yellowish dust on scraping. No crystals.

24./

24. Black cloth stained with decomposing Ox blood May 1908. No stiffening of cloth. Surface shiny. On scraping, a few dark granules obtained. No crystals.

26. Woollen garment stained with CO blood February 1913, exposed in window for three months. Brick red dust from surface gives crystals readily.

27. Human blood on woollen cloth - three months exposed. Crystals readily.

27A. Film of fresh Ox blood so thin that the individual cells were readily counted. Exposed eight days in window. Crystals at once. Scanty, but large and well formed.

Effect of Heat and Cold.

Samples of sheep and ox blood were obtained from the freezing works, which had been exposed to a temperature of 12° below freezing point for several months.

Crystals were obtained readily in every case.

25. Blood on paper heated until paper was charred. No crystals obtained after prolonged treatment.

28. Old Human blood exposed to following temperatures for 5 - 10 minutes.

29. 110 C Good crystals obtained readily.

30. 120 C Atypical crystals, mostly seed-like and dumb-bell shaped.

31. 130 C Few crystals.

32./

32. 140 C Mostly seed-like crystals.

150 C Crystals excellent and obtained readily. (Doubtful about temperature.)

33. 160 C No crystals after repeated trials.

34. 170 C Crystals.

35. 190 C Crystals.

36. 200 C Crystals.

43. Sample of blood from Dublin Abbatoirs which had been converted into Artificial Manure by Strype's process. Exposed in Laboratory for fifteen years. Grey gritty powder. No crystals obtained. (Spect. of Acid Haematin.)

47. Knife lent by Dr. Mason of Hull. Thrown into fire after murder of child. Handle completely burnt off. The blood on the blade looked like a smear of varnish. No crystals obtained after many experiments.

51. Fresh Ox blood heated to 160 - 170°C for thirty minutes. Crystals readily - many atypical with brush-like ends.

52. Ditto heated for one hour at same temperature. Crystals obtained.

Effect of various Chemical Substances.

Absolute Alcohol.

Thin film on glass exposed to light and heat for seven days then treated with Absolute Alcohol.

37. After 24 hours in contact - crystals readily.

39./

39. After 4 days in contact - crystals readily.

" 14 " " " " "

38. Blood from pericardial haemorrhage ten years in Formalin, then dried hard.

+Acetic, Corpuscles distinct and regular, no diminution in size. No crystals could be obtained by any process.

Alkalis.

48. Blood from stomach in a case of Ammonia poisoning. Gives spectrum of alkaline haematin. Nh_4 HS = Haemochromogen. A portion dried gives haemin crystals readily.

53. Cloth smeared with human blood in 1908, soaped, steeped in soapy water, and dried. Crystals readily obtained.

54. Cloth treated with fresh Ox blood, dried in incubator at 37, washed with strongly alkaline soap and water. A quantity of soapy water left in it and dried over the radiator.

A thread of the cloth, treated directly, gave abundant crystals.

55. Blood stained cloth washed with Sol. Ammonia and dried. Crystals readily.

56. Drop of blood mixed with drop of 30% NAoH and dried. Vigorous evolution of CO_2 on addition of acid from Carbonate formed. No blood left.

Acids/

Acids.

Blood treated with 1 - 10 sulphuric acid. No crystals.
" " " very dilute " " " "

Anilin Dyes.

60. Strong watery solution of Bismark Brown mixed with ox blood and dried.
After 24 hours, abundant crystals readily.
" 3 months, " " "

61. Gentian violet, saturated solution in alcohol, excess of this mixed with one drop of blood and dried.
Crystals beautifully distinct on purple background.
After three months, Crystals readily obtained.

63. Saturated watery solution of Eosin.
Crystals readily.
After eight days exposure, crystals readily.
" three months " " "

64. Saturated Basic Fuchsin (in Carbolic, 2%, solution).
Crystals obtained readily.
14 days later "
21 " " "

65. Haematoxylin Solution. (Blood clot tough and tenacious. Crystals readily.

66. Loeffler's Methylene Blue. Beautiful crystals.

67. Saturated Thionin Blue in 2% Carbolic.
Crystals readily and in abundance.
Crystals seem, if anything, to be more easily obtained/

obtained from mixtures of dyes with blood, and are are more easily observed against the coloured background of the dyes not decolourized by the Acetic Acid of the reagent.

Action of Iron. (See 3. 5. 9. 10. 12. 17.)

Crystals obtained from above steel weapons, all of which were rusty, and had been exposed for periods varying from five to thirty-six years.

72. Very rusty Iron smeared with blood, and exposed in window.

24 hours	- Crystals	readily	obtained.
3 days	"	"	"
8 "	"	"	"
14 "	"	"	"
3 weeks	"	"	"
3 months	"	"	"

In this case the blood formed a distinct layer on the surface, and the particles tested were carefully removed with a needle.

48. Blood intimately mixed with Iron Rust and exposed.

24 hours - Crystals readily.

3 days - No crystals after prolonged treatment.

69. Liq. Ferri Perchlor. Fort (Acid Reaction) smeared on blood stained rag and dried. <u>No crystals.</u>

70. Liq. Ferri Perchlor, (diluted). No crystals.

71. Ox blood mixed with solution of Ferrous Sulphate (neutral).

Dried/

Dried fragment gives crystals readily.

1 day in contact " "

2 " " " " "

3 days, 5 days, 10 days, 14 days, 3 weeks, ditto.

3 months - Crystals in abundance.

In the liquid forms of Iron it is the acidity which prevents the reaction. Neutral solutions have no effect. With Ferric Oxide (Rust) there is no change if the blood be in a distinct layer, but if the blood is intimately mixed with the rust no crystals can be obtained.

VARIOUS.

Leather. Smeared with old human blood and exposed. Scrapings give crystals readily in 24 hours. After eight days crystals are obtained with difficulty. After three weeks no crystals could be obtained. A decoction of the leather was distinctly acid.

50. Blood mixed with Tannic Acid.

Crystals readily obtained.

50A. Tannic Acid precipitate after two weeks.

Crystals readily.

It is probably not the tannin in the leather, but the Acid which prevents the formation of crystals.

46. Blood in intestine of Living Pediculus Corporis from clothing three days after receipt of same.

Crystals readily obtained.

Haemin crystals in intestine of Pediculus Corporis. (x 80)

Under higher magnification.

49. Pulex Irritans and Pulex Canis.

Crystals readily obtained in blood in intestine.

Pulex Canis (female) showing haemin crystals in intestine. (x 80)

The same under higher power.

45. Sample of Hommel's Haematogen - Exposed in Laboratory for many years. Contains glycerine, flavouring/

flavouring essences, etc. Spectrum of Oxyhaemoglobin obtained from smear on slide. Crystals obtained with great ease after drying.

44. Black Debris from gut of Tick (Ornithodorus). Last fed on Camel's blood in Arabia in September 1913 (about). Spectrum shows fine band well down in Red at 650 and absorption of purple. No crystals could be obtained.

(Note on other tests later.)

Crystals can be obtained from OxyHB. Reduced HB. Carbon Monoxid HB. Alkaline and Acid Haematin, Mathaemoglobin and Haemochromogen: they cannot be obtained from Iron Free derivatives such as alkaline and acid Haematoporphyrin.

The above investigations indicate the readiness with which this test gives a positive result under widely different conditions and prove it to be a valuable positive test for blood. In the literature on the subject one finds statements to the effect that crystals cannot be obtained under many of the above conditions; this is due either to inaccurate observation or to faulty technique. In a successful result three points must be carefully kept in mind:- The stain must be dry; the Acid must be glacial (i.e. Anhydrous); and the preparation must not be overheated. <u>Hammerl</u> (Vierteljahrs f. Gericht Med. 1892, 4. 44.) states that exposure to sunlight or exposure/

exposure to a temperature of 140 - 145°C inhibits their formation. This does not agree with my experience as I have obtained them repeatedly from stains exposed to sunlight for long periods and from stains exposed to much higher temperatures than 145 C. Blondlot states that absolute alcohol prevents a positive result, it appears to have no effect whatsoever. Apart from the experiments quoted, I have frequently dehydrated my films by means of absolute alcohol to save time in drying without bad effect.

Siefort (Vierteljahr f. Ger. Med. 1898. 16. 1) gives an account of the action of Metallic Oxides, especially Iron Rust, on blood. He states that no crystals can be obtained from rusty iron smeared with blood after five days exposure. Many other writers (Richter, Leers, Wachholz, Puppe, Glaister.) agree with him, but as I have pointed out, unless the blood be intimately mixed with the rust, which will seldom or never occur in practice, the crystals can be obtained from a portion of the surface blood. This is most important for a great majority of tests for blood in Forensic Practice will be performed on weapons of various kinds. I am inclined to think, from my experiments, that the change is due to an acidity of the iron compounds in atmospheric air. There is no destruction of the chromogen group of the haemoglobin molecule for the spectroscopic tests can all be obtained with ease.

Dragendorff (Maschka Handbuch der Ger. Med. 1881.) states/

states that Iron Rust has no effect on blood.

Anilin dye stuffs, stated by Leers to prevent the formation of crystals, have no such effect, at least with the typical though limited series of dye stuffs which I used.

Hoffmann (Vierteljahr f. Ger. Med. 1873. 19. 113) states that when stains are washed with alkaline soap they are so modified that they do not yield the crystals.

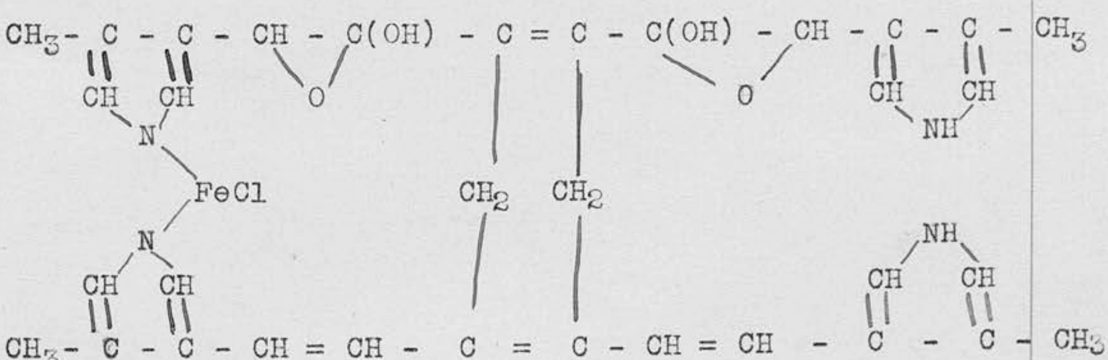
Zanelli (Atti del R. ist. veneto di sci., lett., ed arti 1885. 4. 425.) found that from stains on clothes washed with common soap or the common Italian washing mixture, containing Pot. Carb., crystals cannot be obtained.

Lewin and Rosenstein (Arch. f. Path. Anat. 1895) disagree entirely with this statement and from my own experiments I am of opinion that alkalies have no inhibitory effect. Acids, however, appear to absolutely prevent their formation. If a negative reaction is obtained, however, it is useless to waste time over some of the complicated methods advocated, one should at once proceed to the spectroscopic tests now to be described.

Since Hoppe Seyler first drew attention to the optical characters of Blood Pigment (Arch. Path. Anat. 1892. 23. 446.) the examination of blood stains by this method has attracted much attention amongst Medical/

Medical Jurists. The colouring matter of the blood (Haemoglobin) is a complex conjugated protein which is readily broken down into Globin, a simple protein, and a pigment Haematin which is also relatively simple, by heat, Alkalies, Acids, etc. The latter contains all the Iron of the original haemoglobin .33% (Butterfield, Zeit. Phys. Chemie, 1909 - 62. 173.). If decomposed in the absence of Oxygen then Haemochromogen (and Globin) is formed, which pigment is rapidly again converted into haematin by the action of Oxygen.

Hoppe Seyler showed that the Haemochromogen molecule had the grouping necessary for readily combining with Oxygen and a chromogen group which gave the characteristic spectrum. Haemin may be regarded as the Hydrochloric Ester of Haematin, and according to Franz Müller (Oppenheimer's Handbuch der Biochemie, 1. 720.) has the formula:-



This is the characteristic structure of the

blood/

blood pigment molecule and is only slightly modified in the various derivatives which have been worked out.

Haematin	C_{34} H_{34} N_4 FeO_5	(Kuster)
Haemin	C_{34} H_{33} N_4 O_4 $FeCl$	
Haemochromogen	C_{34} H_{36} N_4 FeO_5	

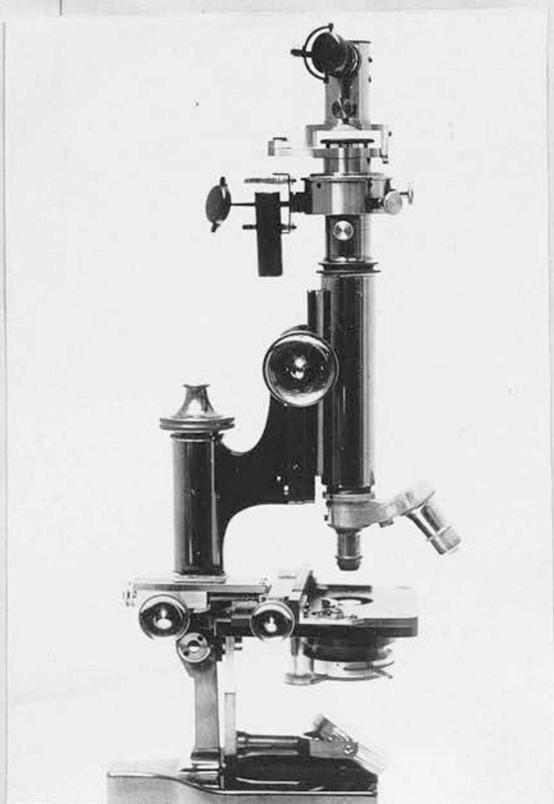
The pigment is extremely stable and IT IS this character together with the readiness with which different spectra can be obtained by the action of various reagents which renders it so valuable as a test for blood, and which distinguishes it from all other pigments.

I do not propose to offer any remarks upon the spectra of Oxyhaemoglobin and reduced haemoglobin, as these are sufficiently well known to everyone, moreover blood changes so rapidly after being shed that there is but little probability of obtaining these characteristic spectra from blood stains in the dry state. Likewise I shall not further mention such derivatives of blood as Haemin, Haematin (Acid and Alkaline), Methaemoglobin, Sulph-haemoglobin, etc., as these pigments give spectra which are by no means delicate, are not characteristic and only serve to confuse.

After extensive examination I have come to the opinion that the following three spectra, namely Haemochromogen, Acid Haematoporphyrin and Alkaline Haematoporphyrin, are by far the most delicate and characteristic/

characteristic, and the most uniformly easy to obtain. Further, the reagents used to obtain these spectra at the same time serve to exclude all other pigments with which blood may be confused. I shall therefore confine myself to a description of the above three derivatives only.

Some form of Spectroscope is necessary to carry out these tests. I have used Zeiss' Microspectroscope, which I illustrate. It is fitted with a comparison prism and wave-length scale and is a most useful instrument. Where a special microspectroscope is not available, a cheap hand spectroscope can be easily adapted by removing the eye piece of the microscope and fitting the hand spectroscope in its place as illustrated. This gives quite satisfactory results.



Zeiss Microspectroscope.

Hand spectroscope fitted to Microscope.

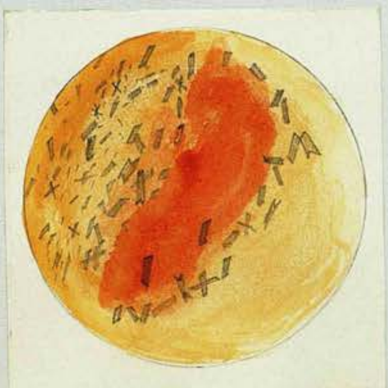
<u>Haemochromogen</u> ($C_{34} H_{36} N_4 FeO_5$) is readily obtained from blood by the decomposition of haemoglobin in absence of Oxygen. It is usually obtained by acting upon a solution of blood with an alkali, then reducing the resultant haematin with Ammonium Sulphide. It is a brilliant red pigment and readily crystallizable. Its spectrum is characteristic, definite and can be obtained from the most minute particle of blood. The method which I advocate has distinct advantages over the older methods inasmuch as no solution of the stain is necessary, the reaction is more delicate and an additional proof can be obtained in the form of crystals.

To a fragment of dust from the stain, or to the residue from Teichmann's test add a drop of the following solution:-

Hydrazin Sulphate (Sat. Watery sol.)	3 parts.
Pyridin Puriss	2 parts.

set aside for a minute or very gently warm.

On examination the fragment is seen to change to a brilliant red colour, and with the high power minute crystals of haemochromogen can be observed.



Drawing illustrating the action of the pyridin reagent on the residue from Teichmann's Test. The red colour seen in the centre is due to the formation of haemochromogen, the haemin crystals from Teichmann's Test are still observed scattered about the field.

These are red in colour and shaped like a cigar, tapering to a blunt point at each end, and having a clear space in the centre like a cigar band. Great numbers of irregular forms will be seen and in some preparations the majority are acicular. They tend to form crosses, rosettes and irregularly formed clumps of crystals. The photograph gives a fair idea of their appearance but the centre clear area cannot be made out.

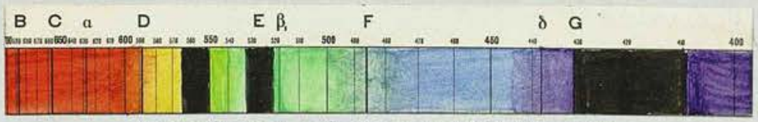
Photograph of Haemochromogen Crystals. (x 480)

Drawing of Haemochromogen crystals showing the clear area in the centre and various shapes of crystals.

Balthazard and Leers consider that this test is more valuable than Teichmann's, but I cannot agree with them, owing to the extreme variability of the crystals, and frequent difficulty of recognition.

If the microspectroscope be now fitted to the microscope, we see the spectrum of Haemochromogen, which consists of two bands. One narrow, dark, distinct band in the yellow (550 - 566) between D and E, the other broader but less distinct in the green (516 - 534), darker/

darker on the left of E and gradually fading as it extends beyond this line. In addition the purple is absorbed from 410 - 433.



Spectrum of Haemochromogen.

I have obtained a positive result with this test in all the experiments quoted under Teichmann's Test. Those specimens which gave no result with Teichmann reacted as follows:-

25. Blood on paper heated until paper was charred. On addition of the reagent a red colour was at once obtained which gave a good spectrum of Haemochromogen.

47. Knife (Dr. Mason of Hull) thrown into fire - handle burnt off. Haemochromogen readily obtained by Pyridin Solution.

43. Sample of Artificial Manure from Dublin Abbatoir (15 years in Laboratory). Red colour at once and good clear spectrum of Haemochromogen.

44. Black Material from Mid Gut of Tick three months after last feed of Camel's Blood in Arabia. Brilliant red colour, Good Spectrum and large Acicular Crystals.

24 etc. Several samples of decomposing blood (advanced). Good spectrum in each case.

38. Blood from Pericardial Haemorrhage ten years in Formalin. Reaction at once.

68./

68. Blood intimately mixed with iron rust and exposed. Reaction moderately good after seven months.

69. Blood stained cotton treated with Liq. Ferri Perchlor. Fort and dried. Red colour and spectrum of Haemochromogen readily obtained.

70. As above with dilute Liq. Ferri Perchlor. Good Reaction.

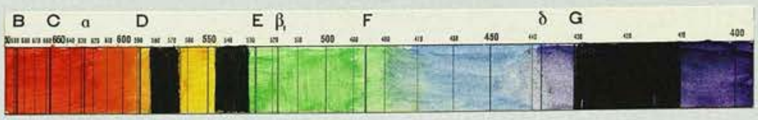
58. Drop of Blood mixed with drop of dilute H_2SO_4. Reaction positive.

41. Blood stained leather exposed for three weeks. Positive.

I have also obtained the reaction from a single thread of blood stained cotton, a particle of blood .4 m.m. in diameter and a film of blood in which the cells were in a single layer.

In the case of Aniline Dyes, 60 - 67, I found that where decolourization of the dye did not take place then there was interference with the spectrum of haemochromogen.

In the case of Carbonmonoxide blood, the reaction is similar but the absorption bands are displaced towards the red end of the spectrum.



Carboxy haemochromogen.

From the above experiments, showing the wide applicability of the test, its delicacy and certainty and/

and the ease with which it can be carried out, one can affirm that it is decidedly one of the safest and best tests for blood.

The Haematoporphyrin Test - Acid Haematoporphyrin.

This is an Iron-free derivative of haemoglobin which was first described by Mulder (Ueber eisenfreies Hämatin, Jour. f. prak. Chem. 1844. 32. 186.) who formed it by the addition of blood to strong sulphuric Acid and called it Iron Free Haematin. It was little used in Forensic practice until Kratter published his experiments in 1892 (Ueber den Werth des Haematoporph. Vierteljahr f. Gerich. Med. 1892. 3. 63.) and though a great amount of work has been done with it on the continent, it is still scarcely mentioned in English works on Forensic Medicine, notwithstanding the fact that its value is equal to that of Haemochromogen and its spectrum quite as characteristic. It is considered by Virchow to be isomoric with Haematoidin, a coloured crystalline produce found in old clots and extravasations. Careful reduction of Haematoporphyrin gives Mesoporphyrin which is very similar to Phylloporphyrin, a derivative of Chlorophyll about which I shall have more to say later. Its chemical structure is almost identical with Haemin with loss of the Iron and addition of two atoms of Oxygen and Hydrogen.

McMunn (Jour. Physiol. Vol.7, No.3.) has demonstrated/

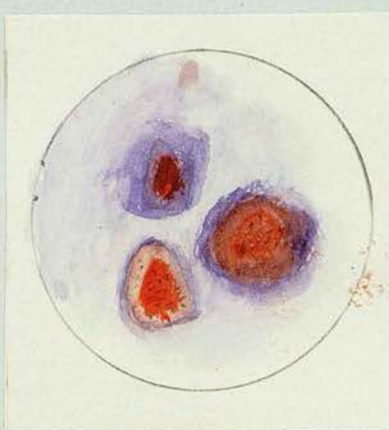
demonstrated its presence under normal conditions in the integument of certain invertebrates - Starfish, Slugs, Lumbricus, in the muscle of Gastropods and in the blood of Annilids. Its function here is doubtful as it does not unite sufficiently easily with Oxygen to be of use as a respiratory pigment.

Nencki, (Oppenheimer's Biochemie 1. 720) says that it can be reconverted into Haematin by treating its ammoniacal solution with Ferrous Sulphate.

Acid Haematoporphyrin can be most readily obtained by the following simple method:-

To a fragment of dry stain, or to the residue from the haemochromogen test add one drop of strong Sulphuric Acid, replace the coverslip and examine.

At once or after gentle warming, the red colour is replaced by a brilliant purple which is characteristic and quite obvious to the naked eye.

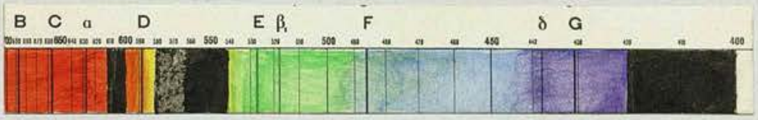


Change in colour observed when residue from haemochromogen test is treated with sulphuric acid.

Examine spectroscopically and the spectrum of acid Haematoporphyrin will be observed.

Two/

Two bands are seen:-



The first, narrow, dark and distinct in the red to the left of D (597 - 612). The second, composed of two parts extending from D into the yellow. 538 - 560 dark and distinct, 560 - 583 much fainter.

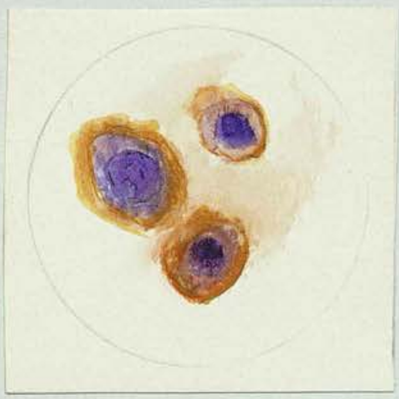
In addition there is absorption in the purple 395 - 420. It is very readily obtained and easily observed. In the case of moderately fresh blood the change is instantaneous, in older stains a few minutes action is sufficient. I have obtained this reaction in every experiment which I have performed, without a single failure.

In old and decomposed blood, such as the black material from the Tick's gut, no purple colour is observed but the spectrum is unaltered. In stains on fabrics where a portion of the material is taken, the charring of the organic matter presents a difficulty, but careful search in the debris about the fibres will give a positive result. With larger qualtities of blood, Ziemke macerates the stain in sulphuric for 34 hours, filters through glass wool and neutralizes with Ammonia. The resultant precipitate is washed and dried in air, rubbed up with equal parts of Alcohol and Liq. Ammon. Fort., filtered and examined for Alkaline Haematoporph, (Vierteljahrs

f./

f. Gericht. Med. 1901. 22. 231.). I have not yet found a case where this tedious method is necessary. Moreover I have always worked with such minute particles that the question of filtration could not be considered. Another advantage of this test is, that a further proof of the presence of blood can be obtained by converting the Acid into Alkaline Haematoporphyrin.

This is one of the most typical and distinctive tests for blood but it is very little used owing to the elaborate technique advocated by those authors who consider it worthy of mention. It can be obtained with the greatest of ease by the following new and simple method which I have adopted. Wash off the sulphuric acid from the preparation above, drop on one drop of 30% sodium Hydroxide, replace the coverslip and examine. At once the purple colour changes to brownish red.



The action of Sodium Hydroxide on Acid Haematoporphyrin.

On spectroscopic examination one gets the beautifully distinct spectrum of alkaline haemato-porphyrin which consists of four distinct bands in the/

the visible spectrum.

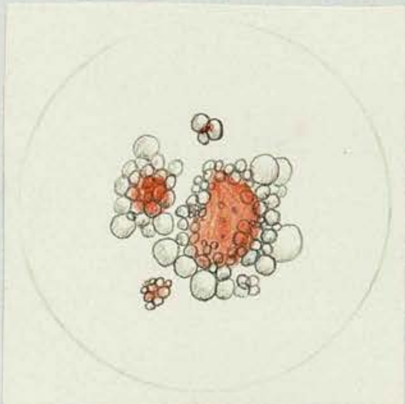
Two between D and E very similar to Oxyhaemoglobin, a dark, narrow band further down in the Red (616 - 633) and a broad distinct band in the green 498 - 521. The purple is absorbed as in the acid derivative 395.- 420. This very marked change in the spectrum with strong acid and strong alkali is one of the most characteristic features of Blood Pigment. It appears that there is more modification than a mere change in reaction, for the Alkaline HP may be strongly acidified with acetic without alteration and even strong sulphuric takes a moderate period before the substance is reconverted into acid Haematoporphyrin.

The above test has given a positive result in every one of my experiments.

<u>Note on Zahn - Gantter and Malachite Green Tests.</u>

In 1871 Zahn (Die Anwendung des Wasserstoff dioxid zum Nachweis von Blutflecken - Corr.- Bl. F. Schweizer Aerzte, 1871. 1. 322.) called attention to the fact that when Hydrogen Peroxide is added to a spot of blood, oxygen is at once liberated actively. The test was lost sight of until again brought forward by Gantter in 1895, who considered it a valuable test. Cotten in 1904 stated that the amount of Oxygen evolved varied sufficiently with different bloods to be/

be of importance in differentiating the source, human blood causing the evolution of more than ten times the amount evolved by the action of sheep's blood. These results have been neither confirmed nor denied. I have used the Hydrogen Peroxide Test in all my experiments and find it a most useful negative test. If a drop of Hydrogen Peroxide be placed on a scraping of the suspected stain and examined microscopically, if there is a trace of blood present, there will be a rapid and vigorous evolution of gas which is seen first at the periphery of each speck of blood, but rapidly becoming evolved from every portion of it, the whole forming a snowy froth.



The action of Hydrogen Peroxide on blood.

The reaction is obtained with bloods exposed to 200°C the evolution of gas here being moderate. In one case (Dr. Mason's knife already alluded to) where the blood had been subjected to very intense heat, I have the following note:-

H_2O_2. Oxygen readily given off from a few particles which were lighter in colour - the black particles give it off much more slowly and in much less amount.

The/

The test is positive after boiling with Acetic Acid, treating with Pyridin, Sulphuric acid, and 30% KOH. Its action depends on a catalytic body (which must be excessively stable) which breaks up the Hydrogen Peroxide. The test is not specific, pus, sputum, etc., also causing a reaction.

Allied to this test and depending on the same property probably is the Malachite Green test first reported by O. & R. Adler in 1904 (Zschr. f. physiol. Chemie. 1904. 59.) and which has been extensively investigated by Lochte and Fiedler (Aerztliche Sachverständigen - Zeitung. 1913. 21. 441.).
The test is as follows:-

.1 gram Basic Leuco Malachite Green (colourless) is dissolved in 25 c.c. of 30% Acetic Acid, made up to 100 c.c. with water, and kept in a dark stoppered bottle.

A few c.c. of this reagent are added to a solution of the suspected stain and then ten drops of Hydrogen Peroxide added. In a short time a brilliant green colour is struck if blood is present.

The test depends on the convertion of the Leuco base into the oxycompound by means of a substance in the blood acting as a carrier from the Hydrogen Peroxide to the dye, the oxy base then being converted into its coloured salt by some of the acids normally present. This being the case, the drawbacks of the test are obvious/

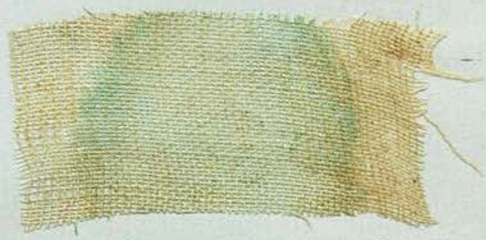
obvious; any oxidising agent or any substance which breaks up Hydrogen Peroxide* will give a positive reaction. The authors are much enamoured of the test, which is certainly a very delicate one, but I cannot agree with many of their statements.

The test is readily obtained with blood diluted to 1.200,000 and with old decomposed blood, blood mixed with Iron Rust, Dyes, Earth, etc.

From haematoporphyrin (slightly), from Haemin crystals, alkaline haematin, Haemochromogen and Methaemoglobin. Blood heated to 160°C. From the Tannic Acid precipitate of CO blood two years old. Sputum alone gives no reaction, but thick phlegm and nasal secretion give a positive result. Old dried semen on cotton gave no result, but a sample of fresh semen reacted readily.

Pus and Vaginal Secretions give a positive reaction. Scrapings from potato give a good positive reaction as do Extract of Red Cabbage, Rhubarb, and Onion.

A Rag smeared with crushed Rhubarb which had the red colour of a blood stain, gave an excellent reaction, although the authors state that vegetable extracts give none.



Rhubarb stain on cotton showing positive result with Malachite Green Test.

Very dilute Potass Permang. reacts readily without H_2O_2, while Pot. Bichrom. requires H_2O_2 before reacting. A sample of colourless serum of an Eclamptic Woman taken ten years ago, and which was decomposed owing to the tube having cracked, gave a very poor reaction, while on spectroscopic examination one could obtain the spectrum of Haemochromogen readily.

Filter paper impressions after Taylor's method from dark clothing, etc., can readily be obtained by saturating the paper with acetic acid and pressing over the stain. The paper is then treated with the reagents and if the stain is blood a beautiful green colour is produced. It reacts well with blood stained urine. If a little of the dye be dissolved in Glacial Acetic and haemin crystals produced in the ordinary way, one can get a confirmatory test by running a drop of H_2O_2 under the coverslip, when the crystals at once turn green. From the few investigations I have made I can affirm that as a Negative Test only, and perhaps for Clinical purposes it is one of the best that have been reported and much superior to the Guaiacum test of Van Deen, but it cannot be looked upon as a specific test for blood.

SUMMARY OF TESTS.

	Specimen.	Haemin Crystals.	HChr.	Ac. HP	Alk. HP.	$H_2 O_2$
1	Meningeal Haemorrhage exposed 25 years	+	+	+	+	not tested
2	From blade of Razor exposed 36 years	+	+	+	+	"
3	From blade of Table knife exposed 8 years	+ scanty	+	+	+	"
5	Fragment .4 mm. diameter from blade of Razor, 11 years (This was mounted permanently and still shows haemin crystals and spectrum of Alkaline Haematoporphyrin after 13 months)	+	+	+	+	+
6	From Bone Handle of same	+	+	+	+	+
7.8	Menstrual Blood, 7 years	+	+	+ not good	+	+
9	Blade of Razor, exposed 6 years	+ atypical	+	+	+	+
10	Razor blade " 15 "	+	+	+	+	+
11	Blood on Wood frozen for several months	+ minute	+	+	+	+
12	Razor blade exposed 14 years	+ abundant	+	+	+	+
13	Old Human Blood exposed on <u>tin</u> for 4 months to sunlight, etc. in window	+	+	+	+	+
14	As above, on <u>Paper</u>	+	+	+	+	+
15	" " <u>Glass</u>	+	+	+	+	+
16	" " <u>Wood</u>	+	+	+	+	+
17	" " <u>Steel knife</u>	+	+	+	+	+
18	Dried fish blood found on street	+	+	+	+	+
19/						

	Specimen.	Haemin Crystals.	HChr.	Ac. Hp	Alk. HP.	$H_2 O_2$
19	Tweed stained with fresh ox blood in 1908	+	+	+	+	slowly small quantity
20	Flannel stained with fresh ox blood in 1908	+	+	+	+	+
21	Green cloth stained with fresh ox blood in 1908. (corpuscles readily made out.)	+	+	+	+	+
22	Blue Vicuna with Human Blood in 1908	+	+	+	+	+
23	Light Homespun with Old Human <u>serum</u> in 1908	-	-	-	-	+
24	Black cloth with decomposing ox blood, 1908	-	+	+	+	+
25	Blood stained paper heated till charred	-	+	+	+	slow but profuse +
26	Woollen garment stained with CO blood exposed 3 months	+	+	+	+	+
27	Woollen garment stained with human blood exposed 3 months	+	+	+	+	+
28	Old human blood exposed for 5 minutes to 110° C	+	+	+	+	+
29	do. do. 120°C	+ not good	+	+	+	+
30	do. do. 130°C	+	+	+	+	+
31	do. do. 140°C	+ seed-like	+	+	+	+
32	Temp. doubtful, all reactions very readily.) 150°C	+	+	+	+	+
33	do. do. 160°C	-	+	+	+	+
34	do. do. 170°C	+	+	+	+	+
35	do. do. 190°C	+	+	+	+	+
36	do. do. 200°C	+	+	+	+	+
51	Fresh ox blood exposed 30 minutes 160° - 170° C	+	+	+	+	+
52/						

	Specimen	Haemin Crystals.	HChr.	Ac. HP	Alk. HP	H_2O_2
52	Fresh ox blood exposed 30 minutes 160° - 170° C	+	+	+	+	+
47	Knife (Dr. Mason) thrown into fire, handle burnt off	-	+	+	+	Slowly
37	Old blood exposed in thin layer on glass in window for 1 month then treated with absolute alcohol 24 hours	+	+	+	+	+
39	Ditto after (a) 4 days, (b) 14 days in absolute alcohol	+	+	+	+	+
38	Pericardial Haem. 10 years in formalin then dried (corpuscles readily made out - normal size)	-	+	+	+	+
41	Leather stained with human blood 8 days in window	+	+	+	+	+
	do. do. 3 weeks in window	-	+	+	+	+
42	Fresh Sheep, Ox, Rabbit, Pigeon, Frog, and Cod blood	+	+	+	+	+
43	Artificial manure from Dublin Abbatoir (15 years in Laboratory)	-	+	+	+	+
44	Black material from gut of Tick Ornithodoras	-	+	+	+	+
45	Sample of Hommel's Haematogen (Exposed in Lab. for years)	+	+	+	+	+
46	Pediculus Corporis from Rape Case (3 days after feeding)	+	+	+	+	+
49	Various Fleas, Pulex Irritans, Pulex Canis and Felis	+	+	+	+	+
48	Blood from stomach in case of Ammonia poisoning	+	+	+	+	+
53	Cloth stained in 1908, scraped and washed in soapy water	+	+	+	+	+
54	Blood stained cloth washed with alkaline soap, quantity of alkaline soapy water left on it, dried over radiator	+	+	+	+	+

	Specimen.	Haemin Crystals.	HChr.	Ac. HP	Alk. HP	H_2O_2
55	Blood stained cloth treated with solution of Ammonia	+	+	+	+	+
58	Blood mixed with dilute sulphuric Acid	-	+	+	+	+
40	Blood mixed with iron rust and exposed outside window for 24 hours	+	+	+	+	+
	Ditto for three days	-	+	+	+	+
68	Ditto for seven months	-	Poor +	+	+	Vigorous +
69	Liq. Ferri Perchlor. Fort (Acid) smeared on blood stained rag	-	+	+	+	moderate +
70	Liq. Ferri Perch. Dil. (acid) smeared on blood stained rag	-	+	+	+	vigorous +
71	Ox blood mixed with Ferrous Sulphate (neutral) and dried	+	+	+	+	+
	In 1 day, 2 days, 3 days, 5 days, 7, 10, 14, 21 days & 3 months	+	+	+	+	+
72	Rusty Iron smeared with blood and exposed in window for 24 hours, not intimately mixed with the rust	+	+	+	+	+
	3, 5, 8, 14 days; 3 weeks; 3 months, ditto	+	+	+	+	+
60	Blood mixed with Sat. Sol. Bismark Brown and dried	+	not	tried		
	ditto after 3 months exposure	+	"		"	
61	Dilute Gentian Violet mixed with ox blood	+	+	+	+	+
62	Saturated alcoholic gentian violet (3 months)	+	on + warming	+	+	+
63	Saturated watery Eosin (a) 1 day, (b) 3 months exposure	+	-	+	-	+
64	Saturated Basic Fuchsin (a) 1 day, (b) 14 days, (c) 3 weeks exposure	+	-	+	+	+

65/

	Specimen.	Haemin Crystals.	HChr.	Ac. HP	Alk. HP	$H_2 O_2$
65	Haematoxylin Sol. (3 months)	+	+	after heating +	+	+
66	Loeffler's Methylene Blue "	+	+	+	+	+
67	Saturated Thionin Blue "	+	+	+	+	+

DYE STUFFS.

The question whether, in the examination of stains directly by the methods indicated above, it is possible to be deceived by the absorption spectra of various dyes - has been raised and is of sufficient importance to merit investigation. In a paper such as the present it would be clearly impossible to deal with even a small percentage of the thousands of dye stuffs, but I have examined a number of representative members of the different groups, many of which are in daily use in dye works, in a pure state, in addition to the investigations I have already noted concerning mixtures of dyes and blood.

Before describing the reaction of the dyes experimented with it will not be out of place to give a short account of the different varieties of dyes and the relation between structure and colour. The synthetic dyes fall into well defined groups according to their chemical nature and are classified as:-

Nitroso dyes	(e.g. Resorcin Green).
Nitro "	(Naphthol Green).
Azo "	(Congo Red).
Triphenyl methanes	(Magenta).
Oxyketones	(Alizarine).
Pyronines	(Eosin).
Diphenylamines	(Methylene Blue).

but/

but in practice they are usually classified according to their method of use disregarding their chemical structure;

e.g. Acid dye stuffs - dyeing wool directly.

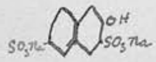
Basic " " - mostly for cotton on tannin mordant.

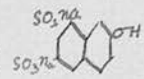
Dye salts " - substantive cotton dyes, (mostly azo dyes).

Mordant dyes, Vat dyes and Developed dyes.

The character of a dyestuff is derived from a group called the Chromophore (which is usually a Quinone group ) the fundamental substance containing the chromophore being called the chromogen. The chromogen is not a dye but is converted into this by the entrance of a salt forming group such as (NH_2) and (OH), these groups are called Auxochromes. For instance Orthophenolphthalein Anhydride is the chromogen of the Phthalein dye stuffs. Though not a dye itself, by the entrance of auxochromes, bases or acids are formed which, when converted into their salts, yield such important dyes as the Eosins, Rhodamines, Fluorescine, Uranine, etc.

The relation between colour and structure has of recent years been clearly established. Many substances which have apparently no colour, e.g. Benzine, Alkaloids, etc., have been shown to give absorption bands in the ultraviolet part of the spectrum. When the Quinone linkage appears in a compound/

compound the intermolecular vibrations seem to be retarded and absorption bands appear in the visible spectrum, hence the Quinone linkage is found in practically all the coloured members of the aromatic series. In the compound itself the position of various groups and their relation to one another have a marked influence on colour. For instance Azo dye stuffs with the Benzine Ring only are yellow, but by increase of the chromophore groups or by increase of the molecular weight the colour deepens to Red, Violet, etc. The effect of the position of the groups is well seen in the Bnaphthol Sulphuric Acids. Where the sulphuric group is in the 2 - 3 - 6 position () the colour is red.

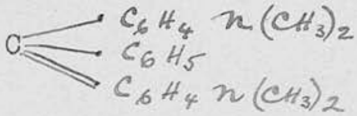
When in the 2 - 6 - 8 position () the colour is yellow.

The position of the Azo groups in the coupling Benzine Ring has a most marked influence on the colour in the tetrazo compounds, for instance the dye-stuff $\begin{matrix}(SO_3NA)_2\\ B(OH)\end{matrix}\ C_{10}H_4N : N - C_6\ H_4N : N - C_{10}H_4\ \begin{matrix}(SO_3NA)_2\\ (OH)B\end{matrix}$ in <u>blue</u> when the two Azo groups are in the <u>para</u>-position, <u>red</u> when in the <u>meta</u>-position.

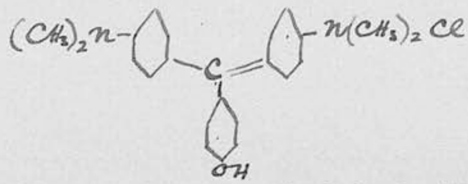
Similarly in the Trephenylmethane series, Magenta having the structure $C \begin{cases} C_6H_4\ NH_2 \\ C_6H_4\ NH_2 \\ C_6H_4\ NH_2Cl \end{cases}$ is brilliant red. If now the (H) of the amido groups be replaced by (CH_3) we get a violet dye, Hexamethyl magenta (or crystal violet) $C \begin{cases} C_6H_4\ N(CH_3)_2 \\ C_6H_4\ N(CH_3)_2 \\ C_6H_4\ N(CH_3)_2Cl \end{cases}$

Further/

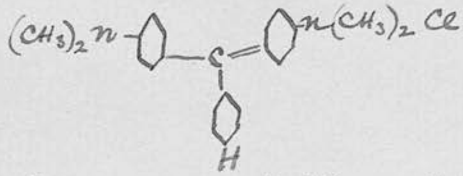
Further, if one of the amido groups of Hexamethyl magenta be eliminated, the violet colour changes to green, giving the compound Malachite green,



Neutral, Acid and Alkaline salts, which have the same combination, may give various colours and various absorption spectra. Thus alcoholic solution of P-oxy-malachite green is green and shews one absorption stripe.



If the hydroxyl group be converted into H the solution becomes Red Violet and gives <u>two</u> stripes in the spectrum.



Malachite green gives <u>green</u> solutions while its analogue Rosaminchloride which contains the Pyrone Ring gives <u>red</u> solutions. Neitzki, at the conclusion of his investigations, stated that "a displacement of the absorption from Violet to Red corresponds in general with the arrangement Orange, Red, Red-violet, Violet, Blue-violet, Blue, Blue-green, Green, a deepening of the colour tint.

The Spectrum itself moves over with increasing molecular weights of the combinations from Ultra-violet to Red in a regular manner."

From/

From the above observations which could be greatly multiplied, one can see the enormous difficulty in working with synthetic dye stuffs, for their number is ever increasing owing to the ease with which new compounds and new colours are formed. However, they tend to fall into groups and the analysis of their spectra will always give some indication of their nature. Thus solutions of the triamido triphenylmethanes show two unequal symmetrical absorption lines, the Thiazines two unequal and asymmetrical lines, the Alizarines three absorption lines, Azo compounds one or two broad bands and so on. This analysis does not come within the scope of this paper and enough has been written to show the manner in which the colour alters with change in the arrangement of the molecule. A full description can be obtained from <u>Formánek</u> - Untersuchung und Nachweis Organischen farbstoffe auf Spectroscop. wege (3 Vols.) including tables of thousands of spectra.

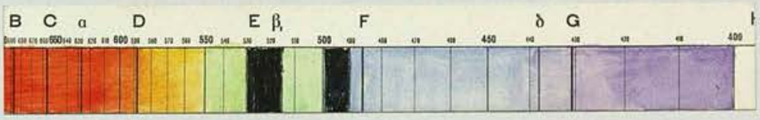
<u>Formanek & Grandmougin.</u> Spectral analytiche Nachweis künstlicher organisher Farbstoffe.

<u>Cain & Thorpe.</u> The Synthetic Dye stuffs.

We are concerned with the more limited question as to whether they could be mistaken for blood; and I give the reactions of a number of the common Red Dye Stuffs tested by the reagents which would be used in a systematic examination of a stain for blood.

Eosin/

Eosin. Pyronine Group (Alkali salt of Tetrabrom fluorescin). Dyes silk and wool weakly acid or neutral. Dyes cotton in tannin mordant. Soluble in water, giving a red solution with slight fluorescence, bands 490 - 500, 515 - 530.



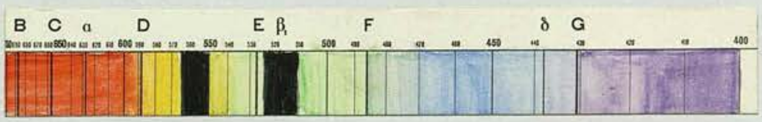
H_2SO_4. Bands in green disappear. Purple absorbed 40 - 43.

KOH NH_4HS. No change.

Pyridin Sol. Bands displaced towards red, 525 - 535.

Erythrosine. Pyronine group. Tetra-iodo fluorescine. Brick red powder readily soluble in water forming a red solution with slight fluorescence.

Two distinct bands in green 510 - 525. 550 - 565, very similar to haemochromogen.



H_2SO_4. Completely decolourized: no spectrum.

KOH. No change.

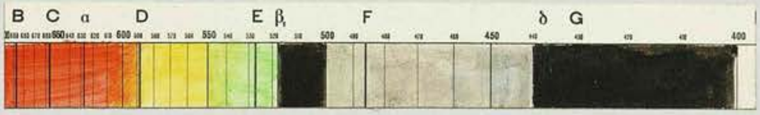
Pyridin. No change, bands clearer.

(KOH and NH_4HS) No change. NB. Haemochromogen similar reaction.

Uranine. Pyronine group. Sodium salt of Fluorescine. Dyes tanned cotton chiefly. Red brown crystals rapidly soluble in water forming yellowish/

yellowish red solution with strong fluorescence.

Dark band in green 500 - 520 shading towards purple, which is completely absorbed.



<u>H_2SO_4.</u> Reddish yellow precipitate and greenish yellow fluid. Dark definite band 480 - 490, gradually growing fainter with time. Sharp absorption of purple 400 - 445.

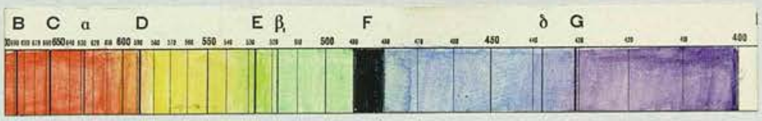
(If added to solid it dissolves with a green fluorescent solution absorbing 400 - 520).

<u>KOH.</u> Unchanged.

<u>Pyridin.</u> Green fluorescence, absorption 400 - 520.

<u>Fluorescine.</u> Pyronine Group.

Red powder readily soluble in water. Yellow green fluorescent solution. Dark distinct band 480 - 490.



<u>H_2SO_4.</u> Brighter green, much less fluorescence. Purple absorbed 400 - 450, distinct and abrupt, no diffuse shading.

<u>KOH.</u> Yellow colour, still fluorescent. Two bands 480 - 490 as before, <u>marked</u>. 460 - 470 faint.

<u>Pyridin.</u> No change.

<u>NH_4OH.</u> No change.

Auramine/

Auramine. Basic dye; used for wool and tannined cotton.

Diphenylmethane Series. Mustard yellow powder, very soluble in water forming a yellow solution. No bands in spectrum. Purple absorbed 400 - 464.

H_2SO_4. Dissolves with evolution of HCl. Completely decolourized. No spectrum.

KOH. Decolourized. Abundant white precipitate. No spectrum.

Pyridin. No change.

Basic Fuchsin. Magenta. Triphenylmethane Series. Readily absorbed by wool. It has little affinity for cotton, a tannic mordant usually being used. It is formed of dark green crystals with metallic lustre. It is readily soluble in water, forming a deep red solution. In a thin layer one sees one band only at 552 - 570 but if a slightly thicker layer be examined, two bands are observed, 480 - 490; 552 - 570, with slight absorption in between.

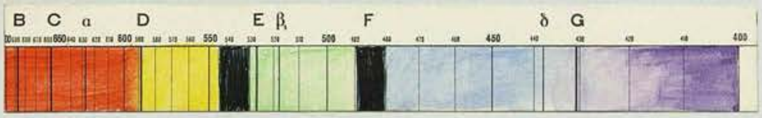
H_2SO_4. Red colour disappears, light yellow. No bands, some absorption of purple.

KOH. Decolourization. No spectrum.

Pyridin. Decolourization.

Acid Fuchsin. Acid Magenta (Triphenylmethane Series). Dark greenish powder readily soluble in water, forming a brilliant red solution with tinge of purple. The spectrum/

spectrum shows two bands, 480 - 490 faint but distinct, 530 - 545 dark and distinct.



<u>H_2SO_4</u> to solution. No change.

To solid. Dissolves, forming an Orange Red solution.

One band 550 - 560 and all purple absorbed 400 - 505.

<u>KOH.</u> Brown then colourless, no bands.

<u>Pyridin.</u> No change.

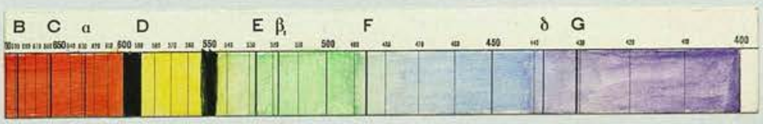
<u>Croceine Scarlet.</u> Acid dye stuff of <u>Azo Group</u> (Disazo). Dyes wool and silk with addition of acid (hot). It is a Brick Red powder readily soluble in water, forming a blood red solution.

Bands 470- 550 and 400- 430 broad and badly defined.

<u>H_2SO_4</u> to solid, purple solution.

Two bands, one at 550 one at 590- 600

Very similar in colour and absorption to Ac HP.



To watery solution - bluish purple. One band 560- 630

<u>KOH.</u> Decolourization. No spectrum.

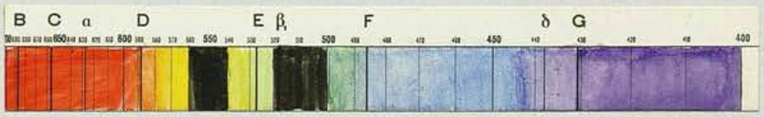
<u>Pyridin.</u> No change.

Diamine/

Diamine Scarlet G B. Direct dye stuff of Azo group (Disazo) constitution uncertain. Dyes cotton with common salt or Glauber's salt. Not readily soluble in cold but readily in hot water. Solution reddish yellow. Spectrum shows two broad indistinct bands, 400 - 430, 470 - 530.

H_2SO_4 to solid, bright blood red solution. Broad band 490- 570, which can be resolved into two 490 - 520, 545- 570.

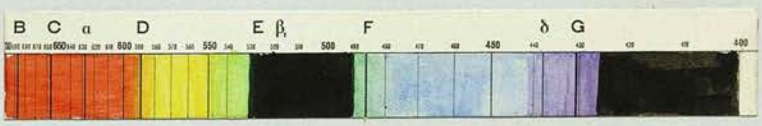
To watery solution, purple-red colour, two bands 500 - 520, 540 - 560.



KOH. Readily soluble, yellow-red solution, spectrum as in H_2O.

Pyridin. Distinct band, 470 - 550.

Sultan Scarlet. 3B. Direct Azo dye, (Disazo). Uncertain constitution. Dyes cotton with NaCl (hot). Soluble in water forming a red solution. Diffuse band, 492 - 531. Purple 400 - 425 and some slight absorption purple to red.



H_2SO_4. Purplish red solution. Two bands 540 - 580, fine band at 600, neither being very sharp.

KOH./

KOH. Precipitate, no spectrum.

Pyridin. No change from H_2O solution.

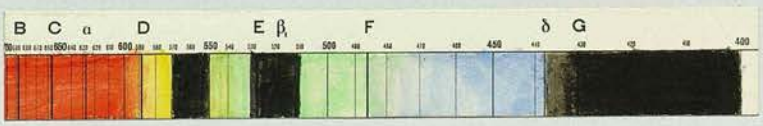
Union Fast Red. Azo Group, (Disazo).

Mixture of direct and neutral, dyeing acid dyestuffs. Dyes cotton, silk and wool with NaCl or Glauber salt. Soluble in water, forming a red solution. Spectrum shows absorption in green, 470 - 582 (560 - 582 very dark). Purple 400 - 441.

H_2SO_4. Bluish purple colour. Absorption 560- 620 400- 435.

KOH. Brown colour and brown precipitate, diffuse absorption of green. No bands.

Pyridin. Two bands 508 - 530, 550 - 572, and some absorption of purple. Note similarity to Haemochromogen.

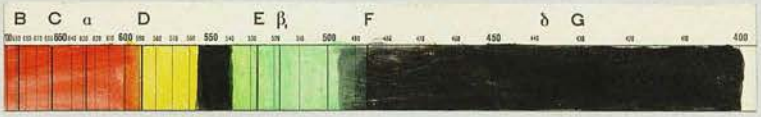


Congo Red. Azo group. Tetrazo compound.

This was the first substantive cotton dye and was first used in 1885, being discovered by Bottiger, and marking a great advance in the application of dye stuffs.

Soluble in water, to a brilliant red solution. Distinct band 540 - 555. Green diffusely absorbed and/

and all the purple.



H_2SO_4 to solution. Bright royal blue colour and black precipitate. Inaistinct band 530 - 570.

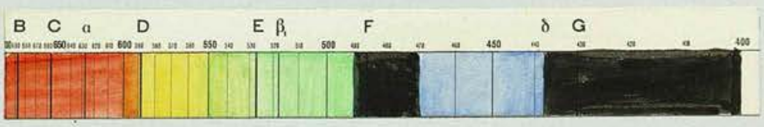
To Solid. Radpidly destroyed - evolution of gas, blue black precipitate.

KOH. Brown precipitate, absorption purple to green. No bands.

Pyridin. Brighter colour with yellowish tinge. Band 482 - 530.

Bismark Brown. Azo Group. Tetrazo compound, (Triamido azobenzine).

One of the earliest of the Azo dyes, (discovered in 1865 by Martias and still very largely used). Basic for wool: no affinity for cotton. It is a dark brown powder readily soluble in water, forming a yellowish brown solution. One band, 472 - 490, and practically the whole of the purple is absorbed.



H_2SO_4 to watery solution. Turns pink with tinge of purple. Dark distinct band 480 - 575. No purple absorption.

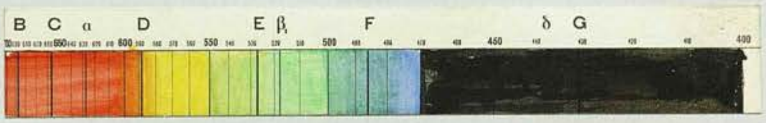
To solid. Dark brown, turning purplish crimson. Dark narrow band 540 - 550. Indistinct band 490. Diffuse/

Diffuse absorption of purple.

KOH. Decolourization, yellowish brown precipitate. No bands.

Pyridin. Unchanged.

Chrysoidine. Azo group (Monazo compound). (Diamidoazobenzine.). Dark crystals with metallic lustre, readily soluble in water, forming a yellow red solution. Purple and blue absorbed 400- 470



H_2SO_4. Red colour, dark band 482 - 523. No absorption of purple.

To solid. Yellow turning red, absorption of purple, some diffuse absorption of blue and green with a darker band 520 - 530.

KOH. Yellow precipitate. No bands.

Pyridin. Precipitate redissolving, solution darker in colour. Purple absorbed 400 - 462.

Tropaeolin. (00) Acid.

Azo dye stuff. Little used. Yellowish red powder, soluble in water, yellow solution. Absorption of purple and blue 400 - 500.

H_2SO_4. Reddish purple colour and absorption of green 470 - 520.

To solid. Absorption of purple 400 - 440 and band as above - 470 - 520.

KOH. Decolourization and yellow precipitate. No bands/

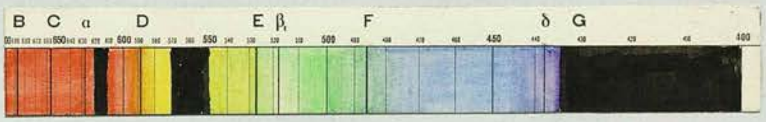
bands.

Pyridin. Unchanged.

Sample 12919 (Baird).

A common red dye stuff of unknown constitution (Secret Formula). Brown powder readily soluble in water, forming a blood red solution. Two bands, 480 - 530, 580 - 600, and complete absorption of violet.

H_2SO_4. Bluish purple colour. Two bands, 550 - 570, 610 - 620, and purple 400 - 435. Similar to Acid Haematoporphyrin but displaced towards red.

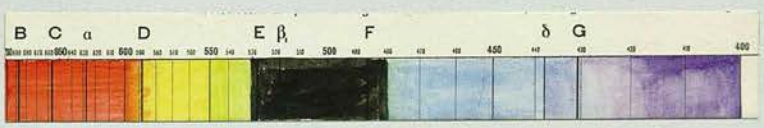


KOH. Brown turning colourless. No spectrum.

Pyridin. No change.

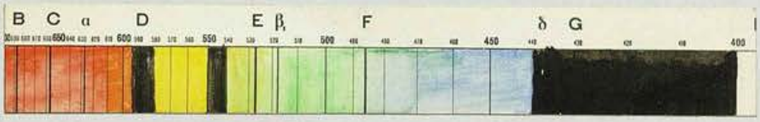
Sample G D. (Baird).

Common red dye stuff (secret formula). Brown powder readily soluble in water forming a bright red solution. One band 485 - 530.



H_2SO_4. Purple colour. Two bands, 540 - 550, 580 - 595. The colour of the solution and the spectrum simulate acid/

acid haematoporphyrin.



KOH. Colour destroyed.

Pyridin. No change.

Saffranine. Diphenylamine dye stuff (Azine group). Basic dye, dyes cotton red on tannic mordant. Soluble in water, forming a red solution. The spectrum shows one broad band from 462 - 520.

H_2SO_4. To solid. Brilliant green solution, on dilution turns to purple then red. Green solution gives diffuse absorption in purple and red. Purple red solution gives sharp band in red 562 - 610.

KOH. Decolourization.

Pyridin. Solution turns crimson with tinge of purple. Sharp distinct band 510 - 550.

Thionin Blue. Diphenylamine dye stuff (Thiazine group). Freely soluble, giving a purplish blue solution. Two definite bands are observed in the spectrum, 560 - 570, 600 - 615, and slight absorption of the purple.

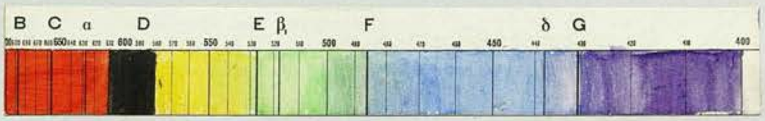
H_2SO_4. Colour changes to light blue, no absorption in/

in spectrum.

KOH. Decolourization, brown precipitate. No absorption in spectrum.

Pyridin. Solution turns pale blue. Black distinct band 600 - 620. Distinct band 680 - 700.

Induline Black. Diphenylamine dye stuff (Induline group). Brown black powder, insoluble in water. Readily soluble in alcohol forming a dark blue solution. Broad band, 582 - 610.



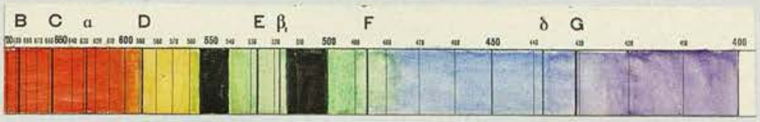
H_2SO_4. Readily soluble, forming a blue solution. Three narrow bands are observed in the spectrum, 635 - 650 sharp black, $\left.\begin{matrix}585 - 600\\545 - 560\end{matrix}\right\}$ not so sharp, and absorption of the purple, 400 - 441.

KOH. Insoluble. Precipitation from alcoholic solution. No spectrum.

Pyridin. Readily soluble. Blue solution. Spectrum as in spirit solution.

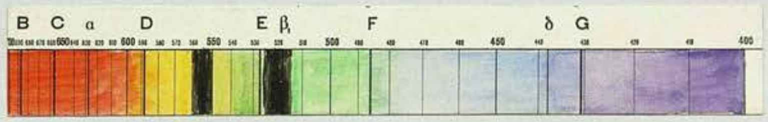
Carmine. Natural pigment extracted from the dried fecundated female insect Coccus Cacti. Red powder insoluble in water, soluble in Sol. Borax, solution having pinkish red colour. Spectrum shows two distinct/

distinct bands, 500 - 515, 540 - 555.



<u>H_2SO4.</u> Colour changes to orange red and one diffuse band 480 - 530 is seen and much absorption of violet; with dilute solution two bands at 500 and 530 can be made out.

<u>KOH.</u> A bluish purple colour is obtained and two bands 515 - 525 and 550 - 560.

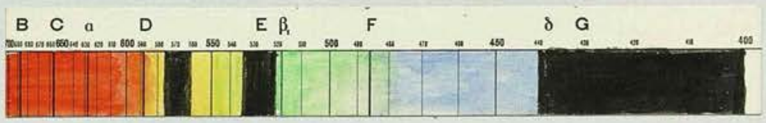


N.B. Similarity to Haemochromogen.

<u>Pyridin.</u> No change.

<u>Picrocarmine.</u> Brownish red solution. Two bands, 522 - 535 and 560 - 575 and purple 400 - 440.

Very similar to haemochromogen.



<u>H_2SO_4.</u> Spectrum displaced towards purple, bands 510 and 550. No absorption in purple.

<u>KOH.</u> One band only 562 - 602 and 400 - 441 in purple.

(NH_4HS. Bands disappear.)

<u>Pyridin.</u> No change.

<u>Haematoxylin.</u> Extract of wood of Haematoxylum Compeachianum. Reddish solution with tinge of purple.

Spectrum shows diffuse absorption of purple to green/

green 400 - 562. No sharp bands.

$\underline{H_2SO_4}$. One band only in green 493 - 540.

<u>KOH.</u> Purple-blue colour and band in red 580 - 623.

NH_4HS. Complete decolourization.

NATURAL PIGMENTS.

Fruit and Grass Stains.

Owing to the manner in which the juices of fruits may simulate blood stains I have investigated the conduct of a number of common fruit juices when treated with the reagents mentioned under the examination of blood. I have examined practically all varieties of fruit and in no case could there be the faintest chance of mistaking such stains for blood. I give the details of Chlorophyll, which has a most interesting spectrum, and a few of the common soft fruits, as these may be of some use in Public Health Work.

Chlorophyll. Found in all the green parts of plants and in greatest quantity in the leaves. It is also found in some animals such as certain worms, Planariae and Coelenterata (Hydra Viridis). These seek sunlight and give off oxygen, but soon die in the dark. Engelmann (Pflügers Arch. Vol. 32, p.80, 1883.) has shown that Vorticellae contain chlorophyll diffused in their plasma.

I have obtained Chlorophyll reactions from the green parasitic worm of Geranium leaves.

It is a complicated body, containing Lecithin and protein bodies and is intimately related to Haemoglobin, like it requiring the presence of Iron for its formation, although like Haematoporphyrin it contains no iron itself/

itself. It can be extracted from the leaves by means of alcohol, preferably after boiling them with water, or the leaf may be examined directly. It gives a green fluorescent solution which, when examined spectroscopically, shows five distinct absorption bands as follows:-

(1) 650 - 670 Sharp, very black, far down in red.

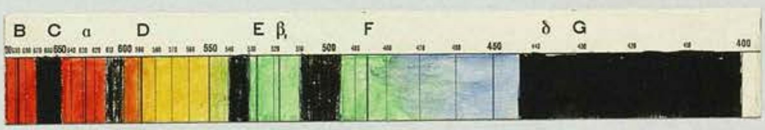
(2) 600 - 615 Narrow, less distinct.

(3) 531 - 540 Plain and distinct.

(4) 495 - 512 Dark but not so sharp.

(5) 400 - 445 In Purple.

The similarity to Alkaline Haematoporphyrin will be at once noticed.



Reactions:-

Pyridin. Lighter and brighter green. No change in Spectrum.

H_2SO_4. No change.

KOH. No change. Bands more distinct.

NH_4OH. No change.

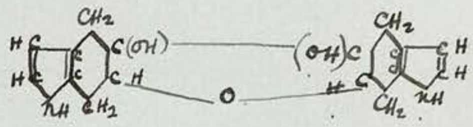
If the alcoholic solution be shaken with Benzine, two pigments are obtained, the blue-green Benzine extract is the same as above with narrow bands in the violet. The yellow pigment left in the alcohol shows only two bands in violet (Kraus). A bye product of chlorophyll called Phylloporphyrin is almost identical with/

with Mesoporphyrin obtained from blood. The chemical composition is almost identical.

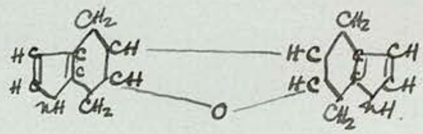
Mesoporphyrin $C_{16}\,H_{18}\,N_2O_3$.

Phylloporphyrin $C_{16}\,H_{18}\,N_2O$.

Mesoporphyrin

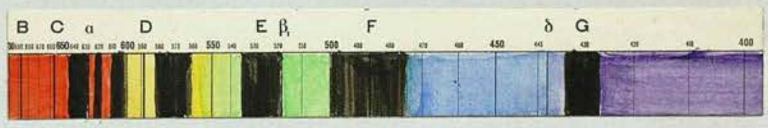


Phylloporphyrin

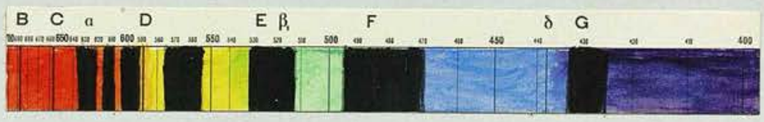


Both on dry distillation yield Pyrrol. Their absorption spectra are closely similar

Mesoporphyrin



Phylloporphyrin



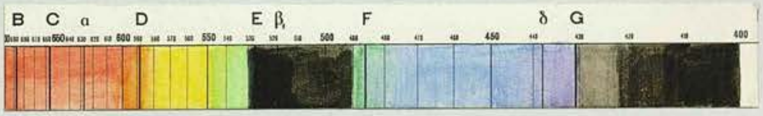
showing the same bands slightly displaced in the plant product to the purple end.

The whole of the chemical and physical characters show the intimate relationship between chlorophyll, the characteristic pigment of the vegetable kingdom, and haemoglobin, the typical pigment of the animal kingdom.

Although it readily undergoes change under natural conditions, I have obtained a perfect spectrum of Chlorophyll/

Chlorophyll in portions of leek examined direct, found in the stomachs of two children who had been immersed in water for over eighteen months, (Winchburgh case).

Strawberry. The watery solution is red in colour, shows only one broad band 490 - 530, and slight absorption of purple.



H_2SO_4 . No change in colour. No change on boiling. Dark band 550 - 540 shading off to 490, (very little change).

KOH. Solution turns orange yellow. Band 490 - 512 and slight absorption of purple.

Pyridin solution. A yellowish purple colour is obtained with diffuse absorption of yellow to purple. No bands.

NH_4OH. Brownish red. One band 562 - 580.

The fruit stain examined direct gave identical reactions.

Solution in alcohol as above.

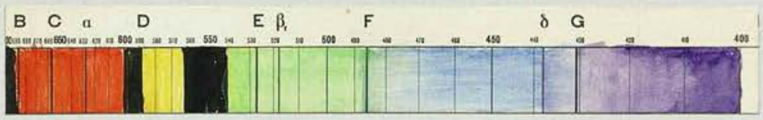
H_2SO_4. Band 480 - 530. Solution more brilliant and tinge of orange.

KOH. Brownish yellow. Band 571 - 590. The colour changes to yellow and the band disappears leaving an absorption of the green - purple.

Cherry/

Cherry. Solution in alcohol, purplish red. Broad indistinct band 541 - 565. Fine distinct band 590 - 600. Absorption of red from 700 - 740.

The colour and spectrum are similar to acid haematoporphyrin.



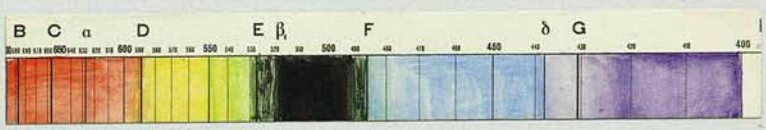
Pyridin. No change. Bands clearer.

H_2SO_4. Colour changes to crimson, dark band 490 - 560, terminating in very dark band at 560.

KOH. Colour changes to green with blue-green precipitate, soluble in excess to green solution. Band at 650. Band at 540. The rest of the spectrum being indistinct. The colour rapidly changes to a golden yellow and the bands disappear.

NH_4OH. Colour changes to a rich blue with a tinge of purple. Sharp band at 600 shading off to 570.

Red Currant. Direct smear on slide. Dark band 490 - 520 with slight absorption on either side.



H_2SO_4. No change. Band sharper.

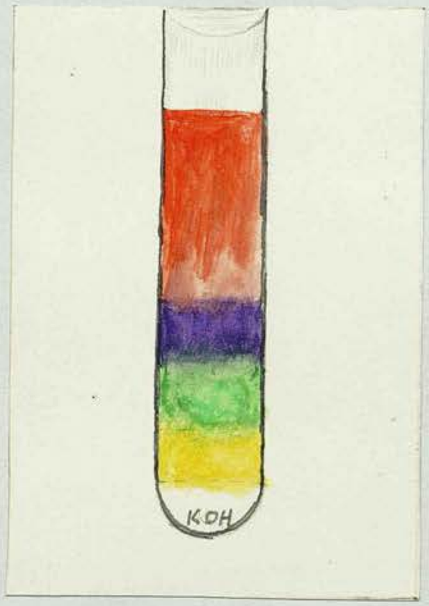
KOH. Gives a very characteristic series of changes. A blue colour gradually appears, changing through grass green to orange, the pulp changing more rapidly than the skin. In green stage there is a distinct band 630 - 640. An indistinct band 561 - 575, and absorption/

absorption of the purple 400 - 471.

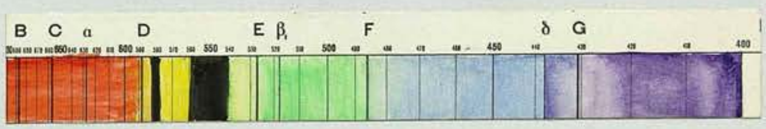
In alcoholic solution the colour is red with a tinge of purple. Dark band 520 - 530 shading off to 510 and 540.

<u>H_2SO_4.</u> No change.

<u>KOH</u> gives a beautiful play of colours, the red changing to purple, then through green to yellow. Spectrum as before.



<u>Pyridin solution.</u> Colour changes to bright purple. Two bands, one at 540 - 560 and a fine band at 580.



<u>NH_4OH.</u> Dark purple colour. Very distinct band 580 - 600.

<u>Black Currant.</u> Direct smear.

Little colour in pulp, mostly in skin and subjacent layer. Band in green 490 - 530 and purple 400/

400 - 442.

H_2SO_4. Colour brighter, no change.

KOH. Light opaque blue changing to bright grass green and finally yellowish green. No bands.

Pyridin. No change.

Watery Solution. Brilliant red colour, rapidly forms into jelly. Well marked band as above, 490 - 530 and some absorption of purple.

H_2SO_4. Colour brighter, jelly becomes fluid. Band as above but brighter and more distinct.

KOH. Colour becomes purple rapidly changing through blue to dark green. Spectrum shows distinct band in red 600 - 620 and diffuse absorption of violet.

Pyridin. Purple colour turning to lilac and finally becoming milky. One band 562 - 583.

NH_4OH. Purple colour changing to bluish green by reflected and red by transmitted light. Distinct band 571 - 613 and some diffuse absorption between purple and green.

In alcoholic solution the reactions are similar.

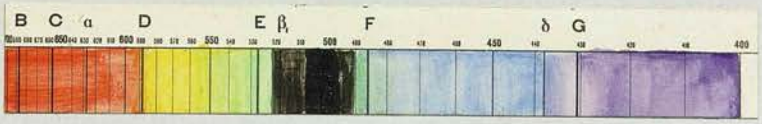
The Ethereal Extract of the berries has a faint green tinge with one distinct fine line of absorption between 630 - 640.

Raspberry. Watery solution.

Blood red solution with dark, well defined

band/

band 490 - 525, darkest at 500.



<u>H_2SO_4.</u> No change.

<u>KOH.</u> Momentary purple, changing through dark bluish green to emerald green. Red by transmitted light. Sharp narrow band 581 - 600, purple 400 - 442. The material becomes gelatinous but soon liquifies, turning yellow at same time.

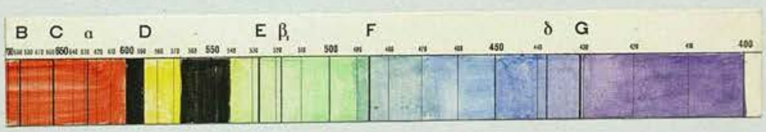
<u>Pyridin.</u> Solution turns purplish red with tinge of blue. Dark band 542 - 560 shading off to 510.

<u>NH_4OH.</u> Bluish green, almost black by reflected light. Reddish purple by transmitted light. Well defined band 500 - 600.

<u>Alcoholic solution.</u> Reactions as above except with Pyridin, with which two bands are obtained, 511 - 561 and fine band at 580.

<u>Red Cabbage.</u> Readily soluble in water, forming a brilliant purple solution.

Spectrum shows a dark band from 542 - 567 and a narrow distinct band from 590 - 600, with some absorption between, very similar to haematoporphyrin.



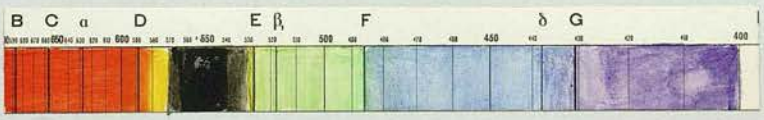
<u>Pyridin solution.</u> The purple changes to blue without altering the spectrum.

<u>KOH.</u> Solution becomes brilliant green, turning yellow with/

with absorption of the purple - no bands. On neutralization a salmon colour is produced which gives a dark band in the green, 491 - 520.

H_2SO_4. Purple colour replaced by red, and spectrum shows band extending from 491 - 520. Solutions in alcohol are much less intense, but the reactions are in all respects similar.

Beetroot. Pigment soluble in water, forming a brilliant red solution. Spectrum shows a single ill defined band in green and yellow, 532 - 571.

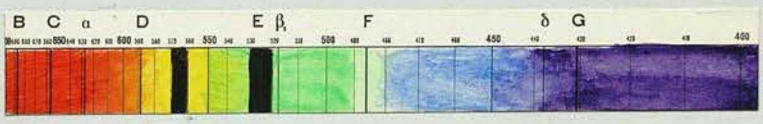


Pyridin. No appreciable change.

H_2SO_4. Colour becomes darker with tinge of purple, the band in the green becoming more distinct.

KOH. Solution becomes brown rapidly changing to yellow. No bands in spectrum. On neutralization, colour and spectrum return.

Tomato. In water a reddish coloured solution is obtained, the spectrum of which shows two bands in the green, 522 - 541 and 561 - 573, similar to haemochromogen.



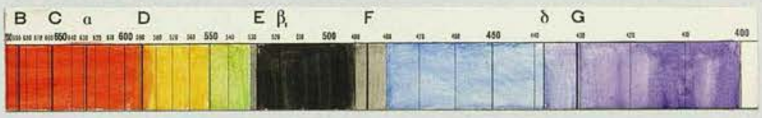
Pyridin. No change.

H_2SO_4. No change.

KOH. Colour turns yellowish green. No spectrum.

Cranberries/

Cranberries. Form a blood red solution in water. Spectrum shows a band extending from 490 - 530 and some diffuse absorption in the green.



Pyridin. Purplish colour with partial decolourization. No bands.

H_2SO_4. No change.

KOH. Solution turns green and shows one fine band in the red at 600.

Technique advised for Examination of Stains.

A few particles of dry stain obtained as above are scraped on to a microscopic slide.

1. Test for haemin crystals in the manner mentioned.

2. Wash off the Acetic Acid by gently dropping distilled water on the preparation by means of a pipette. Then add a drop of the pyridin reagent and examine for haemochromogen.

3. Wash off the pyridin gently with water, allow the preparation to become moderately dry, taking off excess water with filter paper. Add one drop of Concentrated Sulphuric Acid and warm gently. Examine for Acid Haematoporphyrin.

4. Wash off H_2SO_4, add at once one drop of 30% solution of Sodium Hydroxide, allow to stand or warm gently. Examine for Alkaline Haematoporphyrin.

5. Wash off the alkali and add a drop of Hydrogen Peroxide, a snowy froth of bubbles at once appears.

Throughout the procedure the crystals of haematin remain visible.

Method of Mounting Permanent Specimens.

<u>Oxyhaemoglobin</u>, <u>Acid Haematoporphyrin</u> and <u>Alkaline H P.</u> may be mounted in the ordinary way in Canada Balsam and remain unchanged for long periods. Preparations made thirteen months ago in the Department are still unaltered.

<u>Haemochromogen</u> remains for a few months but is gradually converted into Oxy HB as one would expect, when mounted in Balsam or Glycerine Jelly. It can be kept for long periods if mounted in Jelly mixed with Glucose.

C O N C L U S I O N S.

The conclusions derived from my investigations may be summarised as follows:-

1. That by means of the Haemin Crystal Test and the spectroscopic tests the presence of blood can be demonstrated with certainty.

2. That the haemin crystal test gives a positive reaction under a number of conditions, considered by many authorities to be inhibitory.

3. That the following reagent is the best for obtaining the crystals. Potass. Iodid., Potass Bromid. Potass Chlorid. aa grm. .1, Glacial Acetic Acid 100 c.c.

4. That for a successful result the material should be dry, the acetic acid anhydrous, and the heat carefully applied.

5. That the haemochromogen crystal test offers no advantages over the haemin crystal test.

6. That the spectroscopic tests have the widest applicability and give uniformly certain results.

7. That the spectra of Haemochromogen, Acid Haemato-porphyrin and Alkaline Haematoporphyrin are the most delicate and characteristic and are those most readily obtained.

8. That the age of the stain, its insolubility or the conditions to which it has been exposed, such as heat, cold, sunlight, acids, alkalies, preservatives, etc., do not prevent a positive result.

9. That it is unnecessary to dissolve the blood in order to obtain the above spectra, the reagents being applied directly to the dried blood.

10. That the most minute particle of blood is sufficient for the performance of all the tests.

11. That the whole of the tests mentioned can be obtained seriatum from the same particle of blood.

12. That the methods advocated whereby the tests are performed consecutively on the same portion of blood/

blood on a glass slide is of great importance in medico-legal practice and constitutes an advance over the methods hitherto employed.

13. That after carrying out the tests mentioned, the specimen can be mounted and kept indefinitely, as a permanent specimen.

14. That there is no spectrum of blood which cannot be closely imitated by one or other or a mixture of natural or artificial pigments.

15. That no such pigment can give the same sequence of reaction as blood, more especially the reactions to strong acids and alkalies, which have been made practicable by the methods to which I have drawn attention.

16. That mixtures of blood and dyes react well to the haematin crystal test, but cause interference with some of the spectroscopic tests.

17. That dyes fixed on the fibre cause no such interference.

18. That even if spectroscopic examination alone were relied upon, there could be no chance of error if the series of reactions described, be performed.

19. That vegetable colouring matters as a whole remain unchanged under the action of Sulphuric Acid, while Alkalies produce a play of colours ending in green or yellow, which varies with different substances and which readily distinguishes them from blood.

20. That a preliminary test for blood is sometimes of value and for this purpose the Gantter Test (H_2O_2), the Malachite Green and the Guaiac Test of Van Deen are useful, though they cannot be considered specific tests for blood.

21. That the Malachite Green test shows the greatest delicacy of the three and is especially of value if used by the method devised by Taylor of taking filter paper impressions of stains.

22. That its distinctive colour and its power of reacting with dilutions of blood 1 in 200,000 render it not only useful for preliminary tests in medico-legal investigations, but also for testing for blood in urine, stools, etc., in clinical work.

23. That the older chemical and physical tests for blood are of no practical importance and should now receive no further consideration.

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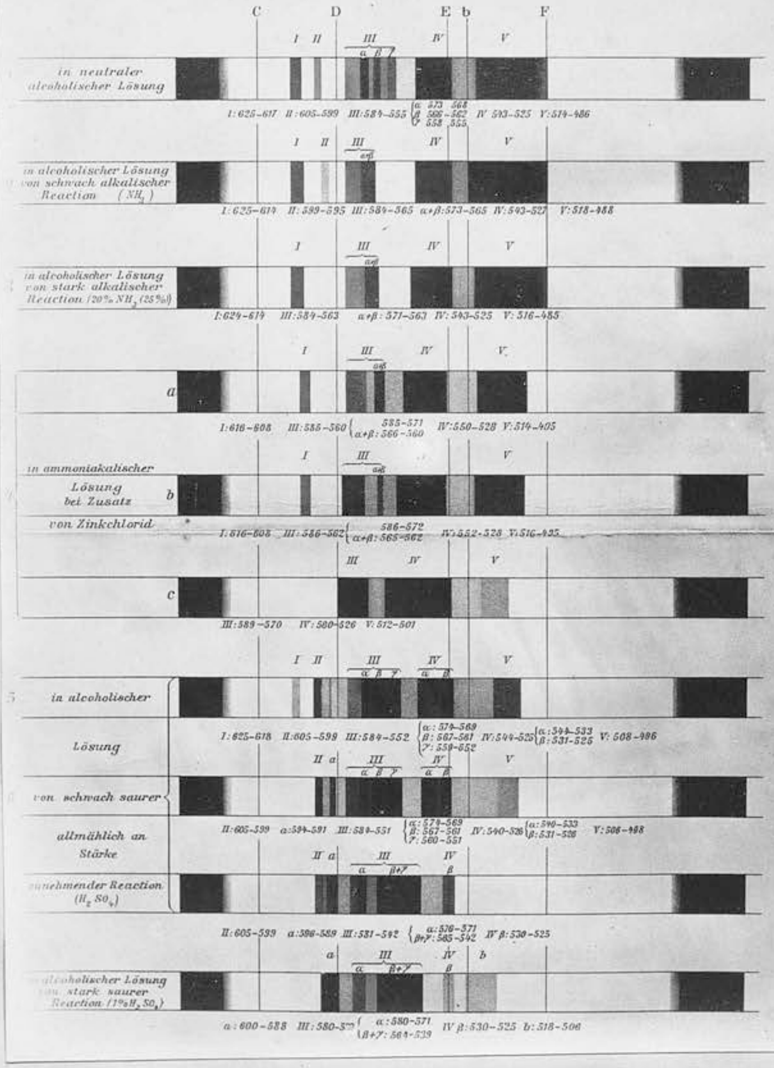

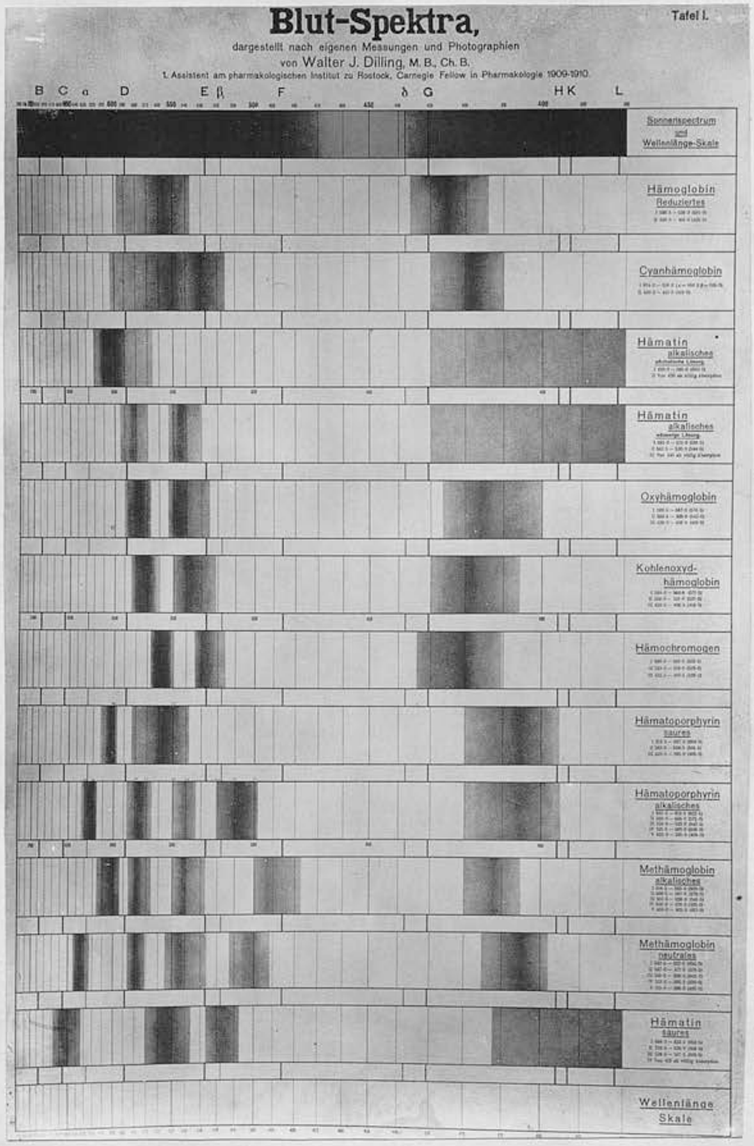
Blut-Spektra,
Tafel I.
dargestellt nach eigenen Messungen und Photographien
von Walter J. Dilling, M. B., Ch. B.
1. Assistent am pharmakologischen Institut zu Rostock, Carnegie Fellow in Pharmakologie 1909-1910.
B C α D E β F δ G H K L
Sonnenspectrum und Wellenlänge-Skala
Hämoglobin Reduziertes
Cyanhämoglobin
Hämatin alkalisches
Hämatin alkalisches
Oxyhämoglobin
Kohlenoxyd-hämoglobin
Hämochromogen
Hämatoporphyrin saures
Hämatoporphyrin alkalisches
Methämoglobin alkalisches
Methämoglobin neutrales
Hämatin saures
Wellenlänge Skale